

Organic Contaminants of Emerging Concern in Land-Applied Sewage Sludge (Biosolids)

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ABSTRACT: Modern wastewater treatment greatly ameliorates the release to the aquatic environment of pollutants present in industrial and residential discharges. However, the recycling of sewage sludge (also known as "biosolids") as a soil amendment presents additional challenges to the wastewater industry, as they must now also assure these complex materials do not adversely effect the environment. Concerns not only include contaminants historically tracked (e.g. heavy metals, petroleum products, PCBs and pesticides), but also those newly discovered in discharges. We recently detected four classes of emerging contaminants (polybrominated diphenyl ethers, alkylphenols and their associated ethoxylates, polycyclic musks, and triclosan) in 12 biosolid samples collected from around the U.S. These findings support the 2002 National Academy of Science conclusion that contaminants of potential toxicological concern, not previously investigated during development of the EPA Part 503-risk assessment, are present in modern biosolids and that a new National Sewage Sludge Survey and updated risk assessment need to be conducted.

INTRODUCTION

HISTORICALLY, wastewater treatment plants (WWTPs) have acted as a readily identifiable conduit for the environmental introduction of toxic chemicals from industrial and residential discharges and urban runoff. However, technological improvements in wastewater treatment processes since the enactment of the Clean Water Act of 1972 have resulted in greatly improved pollutant removal efficiencies. Partitioning of hydrophobic chemicals onto particles and their subsequent removal as sewage sludge is an integral step in the treatment process, decreasing the levels of pollutants discharged to receiving waters. Enhancements in industrial pretreatment have also diminished wastewater burdens of some chemicals before they enter the WWTP. However, despite these improvements recent studies indicate that some poorly studied contaminants of emerging concern may be released through WWTPs. Some of these emerging contaminants preferentially partition into sewage sludge and thus may also be introduced into the environment by land application of sewage

sludge [1, 2]. (Stabilized sewage sludge that is land-applied as a soil amendment or fertilizer is often referred to as biosolids.) While these chemicals of concern are current-use substances, recently detected in the environment, they are currently not regulated in many countries (e.g. not designated as Priority Pollutants in the U.S.). Since the 1988 EPA National Sewage Sludge Survey (NSSS), limited efforts have been made to more fully characterize the range of environmental pollutants of concern within biosolids. However, it has been estimated that over 100,000 chemicals are currently in commerce, with up to 1000 new compounds added each year. Most of these current-use chemicals have not been adequately examined for toxicological or environmental impacts [3, 4]. Preexisting chemicals, previously thought not to present an undue risk, may merit further consideration when new uses or greater demand for them emerges. Also, the discovery of new modes of toxicity or the existence of previously unknown degradation products may merit new concerns to be registered for chemicals previously deemed "safe."

Ideally, environmentally problematic chemicals need to be identified prior to their dissemination into the environment and the potential occurrence of significant damage. But in practice the identification process is of-

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ten reactive, occurring after detrimental impacts have been realized. Some examples of chemicals that have been recognized as environmental problems in the past include; 1,1,1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT), causing decreased reproductive success in raptors [5]; polychlorinated biphenyls (PCBs), with uptake in fishes and subsequent exposure of human consumers [6]; and recently methyl tert-butyl ether (MTBE), a gasoline additive now contaminating some drinking water supplies [7]. Recent surveys indicate additional potentially problematic chemicals are being dispersed into the environment, e.g. into surface waters [8, 9] via WWTP effluents. These include personal-care-products and pharmaceuticals, excreted parent and metabolites, as well as residues released via the deliberate disposal of unwanted and/or expired medicines into WWTP waste streams [8, 10]. Several of these biologically active chemicals have been found resistant to microbial transformation during sewage treatment (e.g., amitriptyline, codeine, and erythromycin) [11, 12]. Pharmaceutical mixtures have been shown to have adverse effects in aquatic ecosystems at dosages much lower than typically taken by humans [13]. Although, drugs receive considerable pharmacological and clinical testing, information on the environmental behavior and ecotoxicity of these biologically active substances and their metabolites are generally not available.

Chemicals that are long-lived, continuously introduced, or subject to wide dispersal are particularly problematic. Remediation of contaminants following their release into the environment is typically much more costly than preemptive routes of disposal (e.g., landfill). In cases where remediation is even possible it may be more destructive to the site than the contaminants themselves. Environmental monitoring efforts have historically focused on priority or historical pollutants [14], using analytical methods that target specific compounds at low environmental concentrations. While these approaches produce useful, accurate and defensible data needed in regulatory affairs, their specificity often obviates the ability to detect the presence of non-targeted chemicals within the environment [14].

Under the 1993 Clean Water Act, the U.S. EPA established regulations for the land application of biosolids with the intent of protecting public health and the environment. The regulation (Standards for the Use and Disposal of Sewage Sludge, Title 40 CFR, Part 503, commonly referred to as the Part 503 rule) sets chemical pollutant limits, operational standards designed to

reduce pathogens and the attraction of disease vectors, and management practices. Compounds considered under this rule were selected based in part on results from the National Sewage Sludge Survey (NSSS), which was designed to detect 411 possible sewage sludge contaminants from 176 WWTPs within the U.S. [15]. Subsequently, nine metals were regulated, primarily due to their recognized toxicity and persistence in soils. Persistence is problematic as it may lead to accumulation of elevated levels over time following repeated applications of biosolids. In the so-called "Round Two" evaluation conducted in 1995 the U.S. EPA revisited the pollutants considered under Rule 503. However, a second comprehensive analytical survey of contaminants in biosolids was not conducted. Instead EPA focused largely on compounds previously considered during the original NSSS, with an emphasis on chlorinated dioxins, furans and co-planar PCBs [16]. In 2003 the U.S. EPA concluded that these compounds did not present a significant risk to human health or the environment and thus will not regulate their levels in biosolids (EPA Headquarters Press Release October 17, 2003). As public health concerns increased regarding the use of biosolids, EPA in 1999 asked the National Academy of Science (NAS) to conduct an independent evaluation reassessing the scientific basis of the Part 503 Rule. In their 2002 report, the NAS committee noted that the Part 503 Rule relied on what is now an outdated biosolid contaminant characterization [17]. Accordingly, due to changes in treatment processes and chemical uses over the last decade, there was concern over possible adverse changes in biosolid composition. The committee recommended that a new national sewage sludge survey be conducted to ensure that Part 503 Rule risk assessment assumptions are based on sound science [17]. At the 2003 Biosolids Research Summit (Proceedings of the Water Research Foundation Biosolids Research Summit 2003) performance of new national surveys of pathogen and chemical constituents of concern in biosolids were ranked as the second and third highest research priorities, following only the development of a rapid incident response study aimed at examining whether a linkage existed between biosolids land application and reports of human health impacts.

Here we report on the presence of four classes of emerging contaminants in biosolid samples obtained from several U.S. states. These include: 1) flame retardant polybrominated diphenyl ethers (PBDEs) used in thermoplastics, circuit boards, and polyurethane foam. Global PBDE demand in 1999 reached 67,125 metric

tons [18]. Like PCBs, 209 different PBDE congeners are theoretically feasible and the same IUPAC PCB scheme is used for their naming. The commercial Deca-BDE (>97% decabromodiphenyl ether, also known as BDE209), constituted about 82% of the reported 1999 global tonnage consumption for all PBDEs. The Octa-BDE formulation makes up 6 % of the 1999 world PBDE market. Hepta- and octa-congeners contribute 70 to 80% of this formulation, with hexa-, nona- and the deca-congeners contributing the remainder. The Penta-BDE formulation constitutes approximately 13% of the 1999 global PBDE market. The vast majority of this, about 98%, is consumed in the U.S. to flame retard polyurethane foams

used largely as padding, e.g. in furniture. Tetra-BDE and penta-BDE congeners (primarily 2,2', 4,4'-tetrabromodiphenyl ether (BDE47) and 2,2', 4,4', 5-pentabromodiphenyl ether (BDE99): (Figure 1, B)) constitute about 80% of this mixture. The remainder consists of 2,2', 4,4', 6- pentabromodiphenyl ether (BDE100), 2,2', 4,4', 5'5- (BDE153) and 2,2', 4,4', 5,6'-hexabromodiphenyl ether (BDE154). These contribute 9.5, 4.5 and 5% of the total, respectively [19]. 2) Alkylphenol polyethoxylate (APEO) degradates, these are formed from surfactants used primarily in heavy-duty detergents. APEOs have been shown to degrade to alkylphenols such as nonyl- (NPs) and octylphenols (OPs) during aerobic and anaerobic pro-

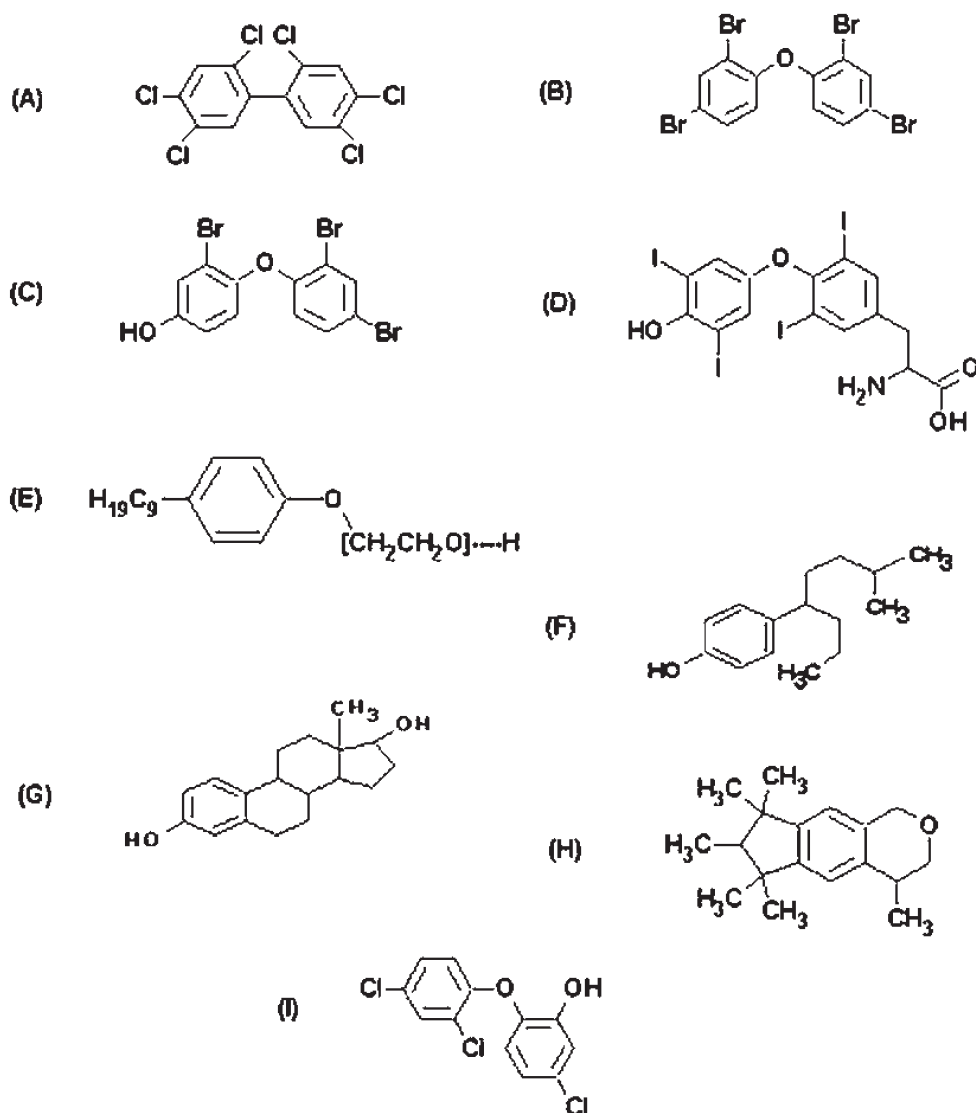


Figure 1. Representative Chemical Structures (A) PCB (2,2', 4,4', 5, 5'-hexachlorobiphenyl, PCB-153); (B) PBDE (2,2', 4,4'-tetrabromodiphenyl ether, BDE47); (C) hydroxylated BDE; and (D) thyroxine, a thyroid hormone; (E) nonylphenol polyethoxylate; (F) a representative 4-nonylphenol; (G) 17β-estradiol; (H) polycyclic musk, galaxolide (1,3,4,6,7,8-hexahydro-4, 6,6,7,8,8-hexamethyl cyclopenta[g][2]benzopyran); (I) triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether).

cesses, such as wastewater treatment. NPs have received recent attention due to their acute toxicity to aquatic organisms and ability to disrupt the endocrine system. The more hydrophobic OP, NP and NP mono- and diethoxylate (NP1EO and NP2EO) degradation products were analyzed due to their greater propensity to sorb to solids. 3) Polycyclic musks common as fragrances, specifically Galaxolide and Tonalide. These have been reported previously to accumulate in fish tissue and human breast milk; 4) anti-bacterial additive triclosan, which is toxic to some aquatic organisms and which may contribute to antibiotic resistance. Triclosan has also recently been reported to photolyze under natural sunlight conditions to 2,8-dichlorodibenzodioxin [20]. Structural similarities of PBDEs and triclosan to PCBs, and NPs to estradiol (Figure 1, B, I, A, and F, G), suggest potentially similar modes of action for these emerging contaminants.

Possible opportunities for lowering contaminant concentrations in biosolids and some additional emerging contaminants, reported by other researchers, will also be highlighted. Finally, we briefly discuss some European regulations, which, unlike current U.S. policies, restrict the use and/or disposal of some of these emerging contaminants of concern.

METHODS

To establish a better understanding of the organic contaminants actually present in contemporary U.S. biosolids, samples produced using different sewage sludge stabilization processes (anaerobic-digested, lime-stabilized, heat-treated and composted) and from

different geographic regions (Table 1) were examined. The analytical approach employed provides the ability to detect targeted compounds, as well as screen complex matrixes for halogenated compounds (e.g., PBDEs and triclosan). A representative list of chemicals detected using this method is listed in Figure 1, (specifically Figure 1: A, B, E, F, H, I).

All samples were freeze-dried, sieved (2000 μ m) to remove large debris and stored in glass jars with Teflon® lids at $<4^{\circ}\text{C}$ until analyzed. Percent solids, Total Organic Carbon (TOC) and Total Nitrogen (TN) were determined for each sample [Table 1]. Percent solids values were determined by heating each sample at 105°C until a constant weight was established. TOC and TN were analyzed by thermal conductivity detection (Exeter CE440, Chelmsford, MA); inorganic carbon was removed by addition of hydrochloric acid. TN includes inorganic and organic nitrogen. Each sample (2–5 g) was subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA). Conditions were: two extraction cycles, pressure @ 1000 psi, temperature @ 100°C , heat 5 minutes, static 5 minutes, 60% flush, purge 180 seconds. Approximately 30 mL of dichloromethane (DCM) were used per sample. Perinaphthenone and 2,2', 3,4,4', 5,6,6'-octachlorobiphenyl (PCB-204) were added as surrogates prior to extraction. Extracts were reduced to 5 mL under nitrogen, and purified by size exclusion chromatography, (Envirosep-ABC®, 350×21.1 mm. column; Phenomenex, Torrance, CA). The column was eluted with DCM at 5 mL/min. The first 50 mL, containing high molecular weight lipids, were discarded. The next 60 mL, containing the compounds of interest, were col-

Table 1. Biosolid characteristics.

Sample	Location (State)	Type of Stabilization	Biosolid Classification	% Solids	% Total Organic Carbon	% Total Nitrogen
Compost-A	VA	Compost	A*	66	9.9	1.3
Compost-B	VA	Compost	A	45	18.5	2.1
Compost-C	TX	Compost	A*	64	16.1	1.6
Lime-A	VA	Lime (Alkali)	B	37	12.3	1.6
Lime-B	VA	Lime (Alkali)	B	31	24.6	2.9
Heat-A	NY/MD	Heat	A*	>95	24.9	4.0
Heat-B	NJ	Heat	A*	>95	32	2.0
AD-A	CA	Anaerobic Digestion	B	30	23.5	3.5
AD-B	CA	Anaerobic Digestion	B	39	22.2	3.8
AD-C	CA	Anaerobic Digestion	B	34	25.4	4.5
AD-D	CA	Anaerobic Digestion	B	44	20.6	3.5
AD-E	CA	Anaerobic Digestion	B	3.0	28.8	5.2

*Biosolids distributed at retail outlets for home garden usage.

lected and solvent exchanged to hexane. The partially purified extract was then introduced onto a 2 g silica column (EnviroPrep, Burdick & Jackson) and eluted with 3 mL hexane, followed by 6 mL of 60:40 hexane/DCM and 10 mL acetone. PBDEs eluted in the second fraction (60:40 hexane/DCM) and OP, NP, NP1EOs, NP2EOs, polycyclic musks, and triclosan were eluted in the third fraction (acetone). Each fraction was collected separately. The retained fractions were reduced in volume and solvent exchanged to toluene. Pentachlorobenzene (0.5 µg) and p-terphenyl (10 µg) were added as internal standards, prior to gas chromatography.

Thereafter extracts were separated on a gas chromatograph (GC) and detected with a mass spectrometer (MS) (Varian Saturn 2000 GC/MS, Sugar Land, TX) operated in the electron ionization (EI) mode with a mass range of 50–650 m/z^+ . The column used was a 60 m DB-5 (J&W Scientific, Folsom, CA) with a 0.25 µm film thickness and 0.32 mm inner diameter. Carrier gas was helium. The GC temperature program used was: initial column setting 75°C, hold one minute, ramp at 4°C/min, hold at 330°C for 5 min., total run time 70 min., injector 315°C. Compounds of interest were quantified with a five-point linear calibration curve using the internal standard and selected ions for each compound of interest, $R^2 > 0.991$ for each calibration curve. Curves were created from 4-tert-octylphenol, a mixture of 4-nonylphenols (Fluka Chemie AG, Switzerland), a NP1EO: NP2EO 60:40 mix (ChemService, West Chester, PA), Tonalide and Galaxolide (Cambridge Isotope Labs, Andover, MA), and triclosan (Ultra Scientific, North Kingstown, RI). Data were corrected based on surrogate recoveries, which were greater than 82 % for perinaphthenone. Selected ions were: OP 135 m/z^+ , NP 135 m/z^+ , NP1EO 179 m/z^+ , NP2EO 223 m/z^+ , Tonalide and Galaxolide 243+244 m/z^+ , triclosan 288+290 m/z^+ , perinaphthenone 152+180 m/z^+ , and p-terphenyl 230 m/z^+ .

PBDEs were initially screened and quantified using a five-point linear calibration curve and internal standard determined on a GC equipped with a halogen-selective electrolytic conductivity detector (ELCD). (PBDE standards included individual congeners, BDE-47, -100, -99, -154, -153 and -209, obtained from Cambridge Isotope Laboratories.) Analytical column type used was the same as stated previously. Carrier gas was helium. The GC temperature program used was: initial column setting 90°C, hold one minute, ramp at 4°C/min, hold at 320°C for 5 min., total run time 68.5

min., injector 320°C. It has been reported that BDE-209 may degrade if subjected to high temperatures for extended periods [21]. Therefore, the extracts were also analyzed on a 15 m DB-5 column of the same film thickness and diameter. Initial column temperature was held 2 min at 80°C, ramped at 15°C / min to 320°C and held for 3 min, then increased at 15°C / min to 350°C for 3 min. Pentachlorobenzene was used as an internal standard and all samples were corrected for PCB-204 recoveries, generally greater than 80%. PBDEs were also confirmed by GC/MS, using the same analytical columns and temperature programs.

RESULTS AND DISCUSSION

Using this screening approach we were able to identify several previously overlooked contaminants of emerging environmental concern in U.S. biosolids. Each sample contained multiple examples of these.

Polybrominated Diphenyl Ethers (PBDEs):

Sources of PBDEs released to the environment are still under investigation. However, they (primarily congeners with six or fewer bromines) have been detected in freshwater fish [22], human breast milk [23] and in biota from remote areas, e.g. deep ocean whales [24] and arctic seals [25], indicating their environmental distribution are tracking that of the PCBs. Hydroxylated-PBDEs are structurally similar to thyroxine (Figure 1, C, D) and triiodothyronine and have been shown to bind to related receptor proteins, suggesting that they may interfere with normal physiological functions [26]. The highly brominated congeners such as BDE209 have primarily been reported from sites near points of release. Their levels in biota have been reported to be low to date, presumed to be largely due to poor uptake efficiency.

PBDEs were detected in each of 11 biosolids analyzed (Table 2) for these compounds. The major PBDE congeners observed were BDE47, BDE99 and BDE209. BDE209 was detected up to 4890 µg/kg (dry wt.). For tetra- through hexa- PBDEs, few differences in concentrations were apparent as a function of WWTP sludge stabilization process used or the facility's geographical location. The commercial penta- formulation (DE-71, Great Lakes Chemical, West Lafayette, IN) in usage in the U.S. today produces a PBDE congener pattern, which closely resembles that detected in each of the biosolids. This suggests that this product, used to-

day exclusively in polyurethane foam, may be the source of lower brominated constituents observed and that little environmental modification had occurred in the mixture's composition, indicating likely proximity to the source (Figure 2). BDE209 used in plastic casings of electronic devices and in latex back coatings on textiles, was the dominant PBDE detected, ranging 84.8 to 4890 $\mu\text{g}/\text{kg}$, dry wt. (Table 2). Although industrial contributions to the WWTP were not characterized, it is believed that major sources of Deca-BDE to the sludge may have been industrial due to: 1) the high concentrations observed; 2) the sample to sample variations in levels seen suggesting point sources; 3) the fact that its dominant use is in physically stable thermoplastics resistant to post manufacture deterioration; and 4) its extremely low water solubility and vapor pressure, limiting environmental movement. However, a recent study from Germany has reported PBDEs in dust samples from 40 households with BDE-209 ranging from 39 to 19,100 $\mu\text{g}/\text{kg}$ [27], indicating that residential dischargers cannot be ruled out. It is believed that the toxicological risk of Deca-BDE is lower than the less brominated PBDEs. Its extreme hydrophobicity and large molecular size likely limits its uptake by organisms. However, it may be subject to debromination, leading to production of lower brominated congeners and other products. For example, in the laboratory, photolysis by UV and sunlight [28] and metabolism exposed fish have resulted in the production of minor amounts of less brominated diphenyl ethers [29]. However, limited documentation of debromination occurring in the field has been published to date.

Deca-BDE is a high-use chemical in the U.S., and is the only PBDE listed on the EPA Toxics Release Inven-

tory (TRI) [www.epa.gov/tri/whatis.htm]. Chemical reporting on the TRI is required if facilities produce more than 25,000 lb or use more than 10,000 lb of a listed chemical. However, there are some exceptions, e.g. a 10 lb threshold for some banned organochlorines and 100 lb for tetrabromobisphenol-A [30], another brominated flame retardant. The latter is not as bioaccumulative as the tetra- and penta- BDEs and is less likely to be released into the environment as it is chemically bonded to its matrix. Lowering the TRI reporting thresholds of all PBDE formulations to a similar level would assist WWTPs and others in identifying sources. Currently there are no U.S. regulations controlling PBDE limits in biosolids. However, due to environmental and human health concerns, the European Union (EU) and California have initiated legislation banning the Penta- PBDE and Octa-PBDE formulations. The EU directive will prohibit these formulations in the EU market by mid-2004 [31]. However, voluntary cessation by industries in many of these countries has already largely curtailed their use there. The phase-out in California is scheduled for 2008 [32]. EU rulings on Deca-BDEs are pending a risk evaluation study, to be finalized by December 2003 [31].

Alkylphenol Polyethoxylate (APEO) Degradates

Recent concerns have been raised about potential estrogenic effects of APEO degradation products. Using the yeast estrogen system (YES) it has been reported that APEOs with the hydroxyl group in the para- position (e.g. 4-NP) interact with the human estrogen receptor (hER) stimulating the synthesis of β -galactosidase

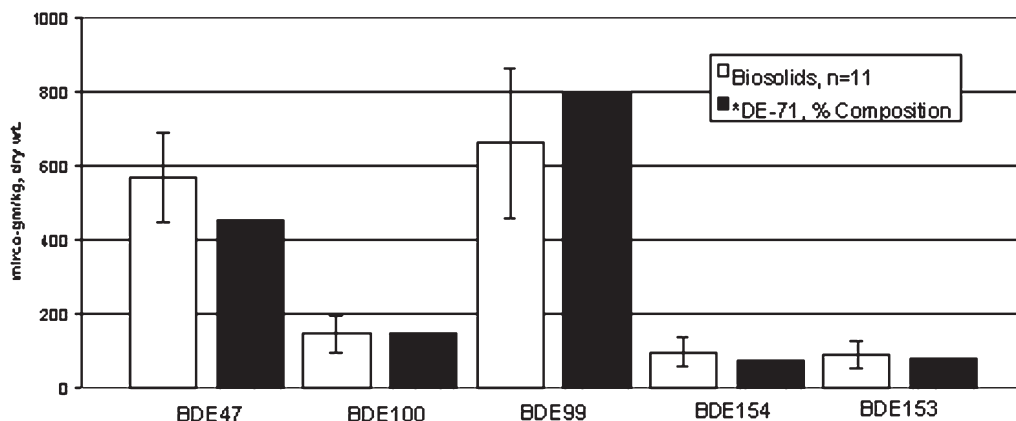


Figure 2. "Penta-BDEs" in Biosolids Compared to DE-71 Composition. (*) Values were derived by multiplying the mean ($n = 11$) of the "penta-BDE" totals (BDE-47, -100, -99, -154, -153) by the % composition of each congener in the commercial formulation DE-71, as reported by Hale, R.C. et al. [22].

Table 1. Emerging chemicals of concern in biosolids.

Sample	Polybrominated diphenyl ethers (µg/kg, dry wt.)					Total Penta-Li ke BDEs	Alkylphenol and nonylphenol polyethoxylates (mg/kg, dry wt.)				Polycyclic musks (mg/kg, dry wt.)		Antibiotic (mg/kg, dry wt.)		
	-47	-100	-99	-154	-153		-209	OP	NPs	NP1EOs	NP2EOs	Total APs & NPEOs		Galaxolide	Tonalide
Compost-A	498	106	743	98.8	55.6	1501	308	<0.5	5.4	0.7	<1.5	6.1	na	na	na
Compost-B	754	167	1157	121	87.9	2287	1460	1.5	172	2.5	<1.5	176	7.0	5.6	7.4
Compost-C	536	112	516	58.2	71.8	1294	368	<0.5	14.2	<0.5	<1.5	14.2	na	na	na
Lime-A	359	88.5	513	82.6	64.3	1107	553	5.3	820	81.7	25.3	932	12.4	7.4	4.7
Lime-B	525	200	584	172	179	1660	84.8	2	119	154	254	529	na	na	na
Heat-A	518	115	714	95.2	58.8	1501	1940	7.5	496	33.5	7.4	544	1.1	0.4	6.9
Heat-B	673	255	815	169	119	2031	4890	na	na	na	na	na	na	na	na
AD-A	605	125	572	57.2	68.9	1428	347	9.9	683	28.4	<1.5	721	17.9	9.0	5.2
AD-B	421	113	391	61	116	1102	340	12.6	720	25.7	<1.5	758	11.4	5.4	5.5
AD-C	686	129	648	61.9	67.7	1593	40	11	779	102	32.6	925	na	na	na
AD-D	674	176	613	74.5	80.6	1618	389	11.7	701	55.8	<1.5	768	na	na	na
AD-E	na	na	na	na	na	na	na	6.7	887	64.9	22.7	981	10.2	6.6	3.6

*na = not analyzed

[33] (Figure 1, F, G). OP, NPs, and NP2EOs have also been reported to induce vitellogenin production (an egg yolk precursor protein, taken up by oocytes to produce proteins) in male trout and in minnows (*Pimephales promelas*) at low $\mu\text{g/L}$ concentrations [34, 35]. Others have reported effects of NPs at concentrations $<1 \mu\text{g/L}$ on developing embryos and larvae of Pacific oysters, including; delays in and abnormal development (e.g. hermaphroditism and shell hinge deformities, and increased larval mortality rates [36]. Wild roach (*Rutilus rutilus*) exposed to discharges from U.K. WWTPs exhibited a high incidence of intersexuality [37]. Concentrations of NPs, NP1EOs and NP2EOs ranged up to $76 \mu\text{g/L}$ in these receiving waters [38]. Reported log octanol-water partition coefficients (K_{ow}) range from 4.12 to 4.48 for these compounds [39], indicating preferential partitioning onto sludge solids and moderate bioconcentration potential.

NPs were the major APEO degradates detected in the U.S. biosolid samples analyzed (Table 2). Concentrations up to 887 mg/kg (dry weight) were observed in anaerobically digested biosolids, but levels in composted samples were considerably lower, 5 to 14 mg/kg . In a recent study by other investigators, anaerobically digested sewage sludges from five wastewater treatment plants in central New York were reported to contain a mean NP concentration of 1500 mg/kg , with a maximum of 1840 mg/kg (dry weight) [40]. Anaerobic digestion of sludge solids has been shown to degrade the parent APEOs, favoring NPs in the final product. NP1EO and NP2EO may also accumulate in sludge solids and may be present at concentrations exceeding NPs [41]. One of our limed biosolid samples (Lime-B, which did not undergo anaerobic digestion) exhibited incomplete APEO degradation, as evidenced by the presence of high concentrations of mono- and diethoxylates, 154 and 254 mg/kg , respectively (Table 2). OP (detected up to 12.6 mg/kg , dry wt. Table 2) was generally two orders of magnitude lower than NP. However, OP has been reported to be 10 to 20 times more estrogenic than NP [41]. Natural and synthetic estrogens (e.g., estrone, 17β -estradiol and 17α -ethinylestradiol) have also been shown to be persistent in activated and digested sewage sludge from Germany, previously reported at up to 37 , 49 , and $17 \mu\text{g/kg}$, respectively [42]. Using the 17β -estradiol equivalence (E2-Eq) factors of 0.0015 and 0.0001 for OP and NP, derived from the YES assay [43], the estrogen activity calculated for these two xenoestrogens identified in our samples set ranged from 0.5 to $98.8 \mu\text{g E2-Eq/kg}$, indicating their

potential estrogenic effects may exceed that of natural and synthetic estrogens within these samples. Also observed within the sample set was a strong positive correlation ($R^2 = 0.6919$) between NP and OP concentrations. Octylphenol polyethoxylates are used in similar applications as NPs, albeit at lower volumes, and likely follow similar degradation pathways. Their presence in sludges suggests that the simple substitution of octylphenol polyethoxylates for NPEOs would not be environmentally advantageous and could actually increase potential xenoestrogen impacts. Comparing stabilization processes (Figure 3), composting and associated aerobic microbial degradation, resulted in the lowest total AP ethoxylates/AP ratios. Composting may also be used to degrade other contaminants susceptible to aerobic degradation.

An EU initiative has set a regulation on the sum of NPs, NP1EO and NP2EO in biosolids at 50 mg/kg , dry wt. The Danish EPA has also set a 30-mg/kg limit for this same sum in biosolids. NPEOs were phased out of domestic detergents in the UK in 1976 and from all usage in Denmark, Germany, Netherlands, Sweden and the UK in the 1980's. In 1987 Switzerland banned the use of APEOs [44]. In contrast, there currently are no North American regulations controlling the usage or disposal of APEOs. However, due to their aquatic toxicity and presence in wastewater effluent APEO-degradation products have been placed on the U.S. EPA TSCA Priority Testing List [45]. Under the Canadian Environmental Protection Act, Canada's EPA has identified NPs and their ethoxylates as "toxic," indicating that further studies are needed to establish their effects on the environment [46]. U.S. EPA is also now considering water quality criteria for NPs at $\mu\text{g/L}$ levels [47].

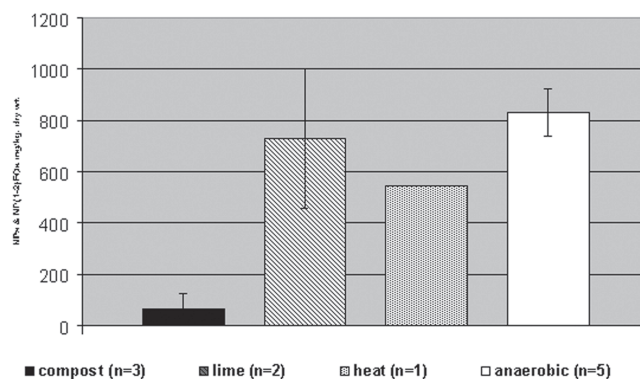


Figure 3. NPs, NP1EOs and NP2EOs totals, grouped by stabilization process.

Fragrance Compounds (nitro- and polycyclic musks)

Nitro and polycyclic musks (Figure 1, H) are used as fragrances in washing and cleaning products and in a variety of personal-care products, including shampoos, soaps, detergents, perfumes and skin lotions. They have been detected in finfish, shellfish and WWTP effluent [48], human milk and adipose tissue [49, 50] and river sediment samples [51]. Log K_{ow} for the nitro-musks, ketone and xylene, range from 4.1 to 5.2, whereas those for the two polycyclic musks, Galaxolide and Tonalide, have been estimated as 5.9 and 5.8, respectively. Bioaccumulation factors (wet weight basis) in trout have been reported as 4200 and 5100 for musk xylene. For bluegill sunfish (*C. dubia*), values of 597 and 1584 were published for Tonalide and Galaxolide, respectively, further underlining their potential to accumulate in aquatic organisms. Concentrations of nitro musks, their amino metabolites, and polycyclic musks have also been previously reported in sewage sludge from 12 Swiss WWTPs [52]. In that study three types of sludges were analyzed and characterized as: highly domestic, mixture (domestic, storm water runoff and low industrial) and highly industrial. The highly domestic sludge actually contained the greatest concentration of musk products, i.e., Tonalide and Galaxolide at 4161 $\mu\text{g/kg}$ and 12,157 $\mu\text{g/kg}$ dry wt., respectively. Nitro-musks, their amino metabolites, and other polycyclic musks were also detected ranging from less than 0.1 to 7, 49 and 843 $\mu\text{g/kg}$, (dry wt.), respectively. Nitro-musks, which are now being replaced with polycyclic musks in Europe, may degrade to amino derivatives during sewage treatment. The latter compounds appear to be more toxic than their precursors.

Tonalide and Galaxolide were detected in each of the six U.S. biosolids, which were analyzed for these compounds (Table 2.). Concentrations ranged from 0.4 to 9.0 mg/kg and 1.1 to 17.9 mg/kg dry wt. respectively, similar to the Swiss data [52]. Sample size was limited, but the data suggest that concentrations in the anaerobic-digested, limed and composted samples did not vary as a function of sewage sludge stabilization. However, levels in the single heat-treated sample (Heat-A) were 10-fold lower. The half-life for polycyclic musks in soil has been estimated to be 180 days [53], suggesting that they could persist in soil after the application of biosolids.

Triclosan

The antibacterial and antifungal agent triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is structurally similar to a hydroxylated-PBDE or PCB, as well as thyroxine (Figure 1, I, C, D). This may indicate some potential to interact with the endocrine system. Triclosan is widely used in personal care products such as shampoos, soaps, deodorants, cosmetics, skin-care lotions and creams, mouth rinses and toothpastes [54, 55, 56]. Reported bioaccumulation factors for zebra fish ranged from 2532 at 3 $\mu\text{g/L}$ to 4157 at 30 $\mu\text{g/L}$ [54]. Fathead minnows, bluegill sunfish, *D. magna* and *C. dubia* all exhibited EC50 or LC50 concentrations in the range of 240 to 410 $\mu\text{g/L}$. Early life stage toxicity for rainbow trout determined a no-observed-effects-value (NOEC) and a lowest-observed-effects-value (LOEC) of 34.1 and 71.3 $\mu\text{g/L}$. However, for the unicellular algae, *A. flos-aquae*, the 96-h EC50 value was only 1.6 $\mu\text{g/L}$ [54]. Triclosan has been shown to be detectable for more than 30 years in anaerobic sediments [57]. It has also been observed in U.S. WWTP influents ranged from 3.8 to 16.6 $\mu\text{g/L}$, corresponding concentrations in final effluents from 0.2 to 2.7 $\mu\text{g/L}$. In aerobic and anaerobic digested sludge concentrations ranged from 0.5 to 15.6 mg/kg, dry wt. The higher levels were present in anaerobic digested sludge solids [55]. It has also been reported that activated-sludge wastewater treatment can remove more than 80% of triclosan from the waste stream and that biodegradation is the likely responsible mechanism. These observations indicate that the type of wastewater and sludge treatment process may influence its elimination [55, 56]. Triclosan was detected in all six biosolids analyzed for this compound (Table 2). Concentrations ranged from 3.6 to 7.4 mg/kg dry wt. There was little apparent association observed between triclosan concentrations and either biosolid stabilization processes or generation facility location in limited sample set examined.

CONCLUSIONS

This survey supports the hypothesis that modern U.S. biosolids contain a diverse collection of wastewater contaminants of emerging toxicological concern not considered by Part 503 Rule. This survey also demonstrates the need to develop analytical methods that can screen for multiply classes of organic contaminants in complex matrixes (e.g. biosolids). Five of the 12 samples were tested for each of the four classes of contami-

nants (PBDEs, APEO degradates, polycyclic musks, and triclosan)—a total of 13 individual contaminants. Two of the five samples contained all 13 contaminants, while the other three samples contained 12 of the 13 (Table 2). Previous studies have shown that sewage sludge can contain a mixture of historically-tracked contaminants (e.g. PAHs, PCBs, organochlorine pesticides). Concerns over possible additive/synergistic effects from biosolids that contain mixtures of contaminants have also been expressed. Researchers using the E-screen test, which measures the response of estrogen-sensitive MCF7 human breast cells, have shown that a combination of weak environmental estrogens can act cumulatively [58]. This suggests that the total estrogen burden or response may be a more toxicologically relevant endpoint to monitor than the concentration of single individual xenoestrogen.

Although these four classes of contaminants were detected in biosolids prior to their land application, questions still remain on their persistence, bioavailability and toxicological effects on terrestrial environments. Further studies on their fate following land application are indicated, as research is limited in this area. Also, these classes of contaminants have previously demonstrated toxicological effects on aquatic environments and there exists a paucity of studies focusing on their terrestrial impacts. Reports have shown that once land applied, decomposition of some biosolid chemical constituents can take place in aerobic environments (e.g. NPs and estradiol [59, 60]), which indicates a potential beneficial process for the elimination of some harmful wastewater residual contaminants. However, uncontrollable events, such as a rain event prior to complete contaminant decomposition, may facilitate transport by leaching contaminants or physically transport biosolid particles with associated contaminant burdens to receiving streams. For example, some researchers have reported APEOs in river sediments and have suggested that they could be derived from sewage sludge used as fertilizer in nearby fields [61]. A change in land use from agricultural to residential, a situation of increasing frequency as a result of expansion of suburbs, may exacerbate human exposure. Further studies on eliminating contaminants in residuals during the wastewater treatment process should take priority; this will ultimately limit harmful exposures.

WWTP technology has primarily focused on removing contaminants from wastewater effluent, thus protecting our waterways from toxic effects to aquatic life or other impacts on downstream use. In contrast, the

anthropogenic fraction of the organic constituents in biosolids, the “second effluent,” has been largely considered benign following the EPA risk assessment and thus has been inadequately studied during the past decade. Interestingly, even the 1988 NSSS examined sewage sludges, not biosolids, an important distinction. Parent xenobiotic burdens in the latter are expected to be lower than in the former. However, in some cases, as illustrated by the NPEOs and NPs, breakdown products may actually be the more problematic. However, some of the contaminants detected in the present study may be reduced through additional stabilization processes, e.g. complete APEO elimination via composting. In other cases, the best approach may be removal of pollutants from the wastewater stream at the source, e.g. collection of unwanted or expired pharmaceuticals from residents prior to their disposal via WWTPs. Further studies focusing on the presence of emerging contaminants in biosolids are obviously indicated, along with efforts to more fully understand their fate during wastewater treatment and sewage sludge treatment and stabilization processes. Chemicals with structures, physical and chemical properties or persistence similar to known problematic contaminants should be of highest priority, as recapture or elimination of these may be difficult if remediation becomes necessary.

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REFERENCES

1. Hale, R. C.; La Guardia, M. J.; Harvey, E. P.; Gaylor, M. O.; Mainor, T. M.; Duff, W. H. (2001a) Persistent Pollutants in Land-Applied Sludges, *Nature*, 412, 140–141.
2. La Guardia, M. J.; Hale, R. C.; Harvey, E. P.; Minor, T. M. (2001) Alkylphenol Ethoxylate Degradation Products in Land-Applied Sewage Sludge (Biosolids), *J. Environ. Sci. Technol.*, 35, 4798–4804.
3. Menditto, A. and Turrio-Baldassarri, L. (1999) Environmental and Biological Monitoring of Endocrine Disrupting Chemicals, *Chemosphere* 1999, 39, 1301–1307.
4. Mumma, R.; Dale, R.; Waldman, J.; Tong, S.; Jacobs, M.; Babish, J.; Hotchkiss, J.; Wszolek, P.; Gutenman, W.; Bache, C.; Lisk, D. (1984) National Survey of Elements and Other Constituents in Municipal Sewage Sludges, *Arch. Environ. Contam. Toxicol.*, 13, 75–83.
5. Fry, D. D. (1995) Reproductive Effects in Birds Exposed to Pesticides and Industrial Chemicals, *Environ. Health Perspect.*, 103 (Suppl. 7), 165–171.
6. Brown, J. F. Jr.; Bedard, D. L.; Brennan, M. J.; Canahan, J. C.; Feng, H.; Wagner, R. E. (1987) Polychlorinated Biphenyl Dechlorination in Aquatic Sediments, *Science*, 236, 709–712.
7. Johnson, R.; Pankow, J.; Bender, D.; Price, C.; Zogorski, J. (2000)

- MTBE - To What Extent Will Past Releases Contaminate Community Water Supply Wells?, *Environ. Sci. Technol.*, 34, 210a–217a.
8. Daughton, C. G and Ternes, T. A. (1999) Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change? *Environ. Health Perspect.*, 107 (Suppl. 6), 907–938.
 9. Halling-Sorensen, B.; Nielsen, N. S.; Ingersiev, F.; Holten Lutzheoft, H. C.; Jorgensen, S. E. (1998) Occurrence, Fate and Effects of Pharmaceutical Substances in the Environment—A Review, *Chemosphere*, 36 (2), 357–393.
 10. Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. (2002) Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999–2000: A National Reconnaissance, *Environ. Sci. Technol.*, 36, 1202–1211.
 11. Richardson, M. L.; Bowron, J. M. (1985) The Fate of Pharmaceutical Chemicals in the Aquatic Environment, *J. Pharm. Pharmacol.*, 37, 1–12.
 12. Rogers, H. R. (1996) Sources, Behavior and Fate of Organic Contaminants During Sewage Treatment and in Sewage Sludges, *Sci. Total Environ.*, 185, 3–26.
 13. Renner, R. (2002) Drug Mixtures Prove Harmful, *Environ. Sci. Technol.*, 268a–269a.
 14. Hale, R.C. and Smith, C.L. (1996) A Multiresidue Approach for Trace Organic Pollutants: Application to Effluents and Associated Aquatic Sediments and Biota from the Southern Chesapeake Bay Drainage Basin 1985–1992. *Int. J. Environ. Anal. Chem.*, 64, 21–28.
 15. Federal Register, (1990) *National Sewage Sludge Survey: Availability of Information and Data, and Anticipated Impacts on Proposed Regulations* (55 FR 47210–47283).
 16. U.S. EPA, (1996) *Technical Support Document for Round Two Sewage Sludge Pollutants*; EPA/822/R-96/003; Office of Water: Washington, DC.
 17. National Research Council, (2002) “Biosolids Applied to Lands: Advancing Practices and Standards,” *National Academy Press*, Washington, D.C.
 18. Renner, R.; *Environ. Sci. Technol.* (2000) What Fate for Brominated Fire Retardants?, *Environ. Sci. Technol.*, 34, 223a–226a.
 19. Hale, R. C.; La Guardia, M. J.; Harvey, E.; Mainor, T. M., (2002) Potential Role of Fire Retardant-treated Polyurethane Foam as a Source of Brominated Diphenyl Ethers to the U.S. Environment, *Chemosphere*, 46, 729–735.
 20. atch, D. E.; Packer, J. L.; Arnold, W. A.; McNeill, K. (2003) Photochemical Conversion of Triclosan to 2,8-dichlorodibenzo-*p*-dioxin in Aqueous Solutions, *J. Photochem. Photobio. A: Chem.*, 158, 63–66.
 21. Kierkegaard, A.; Balk, L.; Tjarnlund, U.; de Wit, C.; Jansson, B. (1999) Dietary uptake and biological effects of decabromodiphenyl ether in rainbow trout (*Oncorhynchus mykiss*), *Environ. Sci. Technol.*, 33, (10) 1612–1617.
 22. Hale, R. C.; La Guardia, M. J.; Harvey, E. P.; Mainor, T. M.; Duff, W. H.; Gaylor, M. O. (2001b) Polybrominated Diphenyl Ether Flame Retardant in Virginia Freshwater Fishes (USA), *Environ. Sci. Technol.*, 35, 4585–4591.
 23. Norén, K.; Meironyté, D., (2001) Certain Organochlorine and Organobromine Contaminants in Swedish Human Milk in Perspective of Past 20–30 years, *Chemosphere*, 40, 1111–1123.
 24. de Boer, J., (1998) Do Flame Retardants Threaten Ocean Life?, *Nature*, 394, 28.
 25. Ikononou, M. G.; Rayne, S.; Addison, R. F. (2002) Exponential Increases of the Brominated Flame Retardant, Polybrominated Diphenyl Ethers, in the Canadian Arctic from 1981 to 2000, *Environ. Sci. Technol.*, 36, 1886–1892.
 26. Meerts, I.A.T.M.; Marsh, G.; van Leeuwen-Bol, I.; Luijckx, E. A. C.; Jakobsson, E.; Bergman, A.; Brouwer, A., (1998) Interaction of Polybrominated Diphenyl Ether Metabolites (PBDE-OH) with Human Transthyretin *In Vitro. Organohalogen Compd.*, 37, 309–312.
 27. Knott, W.; Mann, W.; Meyer, R.; Nebhuth, J. (2003) Brominated Diphenyl Ether in Indoor Dust, *Organohalogen Compd.*, 61, 207–210.
 28. Watanabe, I. and Tatsukawa, R., (1987) Formation of Brominated Dibenzofurans from the Photolysis of Flame Retardant Decabromobiphenyl Ether in Hexane Solution by UV and SUN Light, 39, 953.
 29. Stapleton, in press
 30. Hileman, B. (1999) RIGHT TO KNOW: EPA Proposes Lower Reporting Thresholds for Persistent Bioaccumulative Chemicals, *Chem. Eng. News*, 77, 4.
 31. European Parliament, (2002) “24th Amendment of Council Directive; 76/769/EEC”; Committee on the Environment, Public Health and Consumer Policy. European Union.
 32. Renner, R.; (2003) California first in U.S. to prohibit flame-retardants. *Environ. Sci. Technol. Online News*, September 25, 2003. http://pubs.acs.org/subscribe/journals/esthag-w/2003/sep/policy/rr_retardants.html
 33. Routledge, E. J.; Sumpter, J. P. (1997) Structural Features of Alkylphenolic Chemicals Associated with Estrogenic Activity, *J. Biol. Chem.* 7, 272, 3280–3288.
 34. Jobling, S.; Sheahan, D.; Osborne, J. A.; Matthiessen, P.; Sumpter, J. P. (1996) Inhibition of Testicular Growth in Rainbow Trout (*Oncorhynchus Mykiss*) Exposed to Estrogenic Alkylphenolic Chemicals, *Environ. Toxicol. Chem.*, 15, 194–202.
 35. Harries, J. E.; Runnalls, T.; Hill, E.; Harris, C. A.; Sumpter, J.P.; Tyler, C.R. (2000) Development of a Reproductive Performance Test for Endocrine Disrupting Chemicals Using Pair-Breeding Fathead Minnows (*Pimephales Promelas*), *Environ. Sci. Technol.*, 34, 3003–3011.
 36. Nice, H. E.; Morritt, D.; Crane, M.; Thorndyke, M. (2003) Long-term and transgenerational effects of nonylphenol exposure at a key stage in development of *Crassostrea gigas*. Possible endocrine disruption?, *Mar. Ecol. Prog. Ser.*, 256, 293–300.
 37. Jobling, S.; Nolan, M.; Tyler, C.R.; Brighty, G.; Sumpter, J. P. (1998) Widespread Sexual Disruption in Wild Fish, *Environ. Sci. Technol.*, 32, 2498–2506.
 38. Blackburn, M.A.; Kirby, S. J.; Waldock, M. J. (1999) Concentrations of Alkylphenol Polyethoxylates Entering UK Estuaries, *Mar. Pollut. Bull.*, 38, 109–118.
 39. Ahel, M and Giger, W., (1993) Partitioning of Alkylphenols and Alkylphenol Polyethoxylates Between Water and Organic Solvents, *Chemosphere*, 26 (8), 1472–1478.
 40. Pryor, S. W.; Hay A. G.; Walker, L. P., (2002) Nonylphenol in Anaerobically Digested Sewage Sludge from New York State, *Environ. Sci. Technol.*, 36, 3678–3682.
 41. White, R.; Jobling, S. A.; Sumpter, J.P.; Parker, M. G. (1994) Environmentally Persistent Alkylphenolic Compounds are Estrogenic. *Endocrinology*, 1, 175–182.
 42. Ternes, T. A.; Andersen, H.; Gilberg, D.; Bonerz, M. (2002) Determination of Estrogens in Sludge and Sediments by Liquid Extraction and GC/MS/MS, *Anal. Chem.*, 74, 3498–3504.
 43. Holbrook, R. D.; Novak, J. T.; Grizzard, T. J.; Love, N. G. (2002) Estrogen Receptor Agonist Fate during Wastewater and Biosolids Treatment Processes: A Mass Balance Analysis, *Environ. Sci. Technol.*, 36, 4533–4539.
 44. Cork Institute of Technology, (1999) *D-3 Nonyl phenol & nonylphenol ethoxylate*; Clean Technology Center: Cork, Ireland.
 45. Federal Register, (2000) *Forty-sixth Report of the TSCA Interagency Testing Committee to the Administrator, Receipt of Report and Request for Comments*; Notice, Vol. 65, No. 232, 75552–75561.
 46. Environmental Canada Health Canada (2001) CEPA, 1999, Priority Substances List Assessment Report, Nonylphenol and its Ethoxylates, April 2001.
 47. Great Lakes Environmental Center, (2001) *Ambient Aquatic Life Water Quality Criteria—Nonylphenol, Draft Report: EPA Contract No. 68-C-98-134*; Work Assignment No. 2–22, Michigan, USA.
 48. Gatermann, R.; Biselli, S.; Huhnerfuss, H.; Rimkus, G.; Hecker, M.; Kerbe, L. (2002) Synthetic Musk in the Environment. Part 1: Species-Dependent Bioaccumulation of Polycyclic and Nitro Musk Fragrances in Freshwater Fish and Mussels, *Environ. Contam. Toxicol.*, 42, 437–446.
 49. Rimkus, G. G.; Wolf, M. (1996) Polycyclic Musk Fragrances in Hu-

- man Adipose Tissue and Human Milk, *Chemosphere*, 33 (10), 2033–2043.
50. Zehring, M.; Herrmann, A. (2002) Analysis of Polychlorinated Biphenyls, Pyrethroid Insecticides and Fragrances in Human Milk Using a Laminar Cup Liner in the GC Injector, *Eur Food Res. Technol.*, 212, 247–251.
51. Winkler, M.; Kopf, G.; Hauptvogel, C.; Neu, T. (1998) Fate of Artificial Musk Fragrances Associated with Suspended Particulate Matter (SPM) from the River Elbe (Germany) in Comparison to Other Organic Contaminants, *Chemosphere*, 37 (6), 1139–1156.
52. Herren, D.; Berset, J. D. (2000) Nitro Musks, Nitro Musk Amino Metabolites and Polycyclic Musks in Sewage Sludges Quantitative Determination by HRGC-Ion-Trap-MS/MS and Mass Spectral Characterization of the Amino Metabolites, *Chemosphere*, 40, 565–574.
53. Balk, F.; Ford, R. (1999) Environmental Risk Assessment for the Polycyclic Musks ATTN and HHCB in the EU, I. Fate and Exposure Assessment, *Toxicology Letters*, 111, 57–79.
54. Orvos, D. R.; Versteeg, D. J.; Inauen, J.; Capdevielle, M.; Rothenstein, A.; Cunningham, V. (2002) Aquatic Toxicity of Triclosan, *Environ. Toxic. Chem.*, 21 (7), 1338–1349.
55. McAvoy, D. C.; Schatowitz, B.; Jacob, M.; Hauk, A.; Eckhoff, W. S. (2002) Measurement of Triclosan in Wastewater Treatment Systems, *Environ. Toxic. Chem.*, 21 (7), 1323–1329.
56. Thomas, F. W.; Kaiser, S. K.; Nuck, B. A. (2002) Fate and Effects of Triclosan in Activated Sludge, *Environ. Toxic. Chem.*, 21 (7), 1330–1337.
57. Singer, H.; Müller, S.; Tixier, C.; Pillonel, L. (2002) Triclosan: Occurrence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters, and Lake Sediments, *Environ. Sci. Technol.*, 36, 4998–5004.
58. Soto, A. M.; Chung, K. L.; Sonnenschein, C. (1994) The Pesticides Endosulfan, Toxaphene, and Dieldrin Have Estrogenic Effects on Human Estrogen-Sensitive Cells, *Environ. Health Perspect.* 102: 380–383.
59. Hesselsoe, M.; Jensen D.; Skals, K.; Olesen, T.; Moldrup, P.; Roslev, P.; Mortensen, G., K.; Henriksen K. (2001) Degradation of 4-Nonylphenol in Homogeneous and Nonhomogeneous Mixtures of Soil and Sewage Sludge, *Environ. Sci. Technol.*, 35, 3695–3700.
60. Lee, L. S.; Strock, T. J.; Sarmah, A. K.; Rao, P. S. (2003) Sorption and Dissipation of Testosterone, Estrogens, and Their Primary Transformation Products in Soil and Sediment, *Environ. Sci. Technol.*, 37, 4098–4105.
61. Petrovic, M.; Lacorte, S.; Viana, P.; Barceló, D. (2002) Pressurized Liquid Extraction Followed by Liquid Chromatography-Mass Spectrometry for the Determination of Alkylphenolic Compounds in River Sediments, *J. Chromatogr. A*, 959, 15–23.